PART ONE

FUNDAMENTALS

1

Biological Background

In this chapter, we give a brief biological introduction necessary not only to understand the context of the mathematical models developed in the following chapters, but also to appreciate the relevance of the biophysical problems addressed. This gives us the opportunity to present a short overview of the role played by fluid dynamics in biology.

1.1 The Biological World

A simple illustration of the taxonomy of the biological world is shown in Fig. 1.1. Organisms are classified as belonging to one of two domains: prokaryotes, which are single cells that lack a nucleus and other important organelles, and eukaryotes, whose cells do contain a nucleus. As we explain below, prokaryotes and eukaryotes also differ fundamentally in the way they move in a fluid. The common ancestor of both domains dates back approximately 3.5 billion years, not long after the start of cellular life on Earth about 4 billion years ago.

Each domain is further subdivided into kingdoms, and for each of them we indicate in Fig. 1.1 the orders of magnitude of the numbers of different species they include. The prokaryotic domain is made up of two kingdoms: bacteria, which represent the majority of prokaryotes and whose locomotion is studied in detail in this book, and the much less studied archaea.

In contrast, four eukaryotic kingdoms exist. The first, protists, contains the simplest eukaryotes, and includes both unicellular and multicellular algae, many of which are self-propelled and are discussed in this book, but also protozoa and slime molds.

Next comes the animal kingdom which, with over a million species, has the largest diversity of species in the entire living world. Groups of animals sharing many common traits include sponges, jellyfish, worms, insects, crustaceans, mollusks and vertebrates. Of course a multitude of organisms move in fluids within

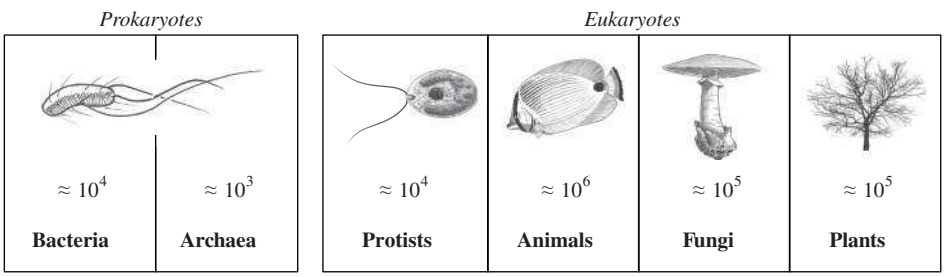

Figure 1.1 Taxonomy of the biological world. Life is divided into two domains, prokaryotes (cells lacking a nucleus) and eukaryotes (cells with a nucleus). Prokaryotes are further divided into two kingdoms (bacteria and archaea) while eukaryotes are typically divided into four kingdoms (protists, animals, fungi and plants). The order of magnitude of the numbers of different species is given for each kingdom. Drawings courtesy of Jacques Lauga.

this kingdom, from self-propelled cells to small insects and large whales. In this book, we characterise in particular the locomotion of spermatozoa, male gametes involved in the sexual reproduction of many animals.

The last two kingdoms involve living organisms that are in general not capable of self-propulsion and are not discussed in this book. Fungi are spore-producing organisms whose digestion takes place outside their body (they include molds, yeasts, truffles and mushrooms), while plants use photosynthesis to produce their nutrients (they include both vascular and non-vascular plants, seed plants, angiosperms and monocots).

1.2 Fluid Dynamics in Biology

Fluids are relevant to all forms of life, from cells to higher organisms, and are involved in virtually all biological processes. Fluids in movement also play important biological roles, in the case of both gases (e.g. air) and liquids (e.g. water). The classical book by Vogel (1996) gives a comprehensive overview of the impact of moving fluids in biology, and it has long been a tradition of fluid dynamicists to develop mathematical models to capture these effects (Lighthill, 1975).

Broadly speaking, biological fluid dynamics problems may be separated into two categories: internal and external problems. Internal fluid dynamics is concerned with fluid motion inside an organism. The two most famous examples of internal problems are blood circulation, which encompasses problems with a range of length and timescales (Pedley, 2000; Popel and Johnson, 2005), and the respiratory system, which includes gas exchange and multiphase fluid physics (Grotberg, 1994; Heil and Hazel, 2011). Another important example at the cellular scale is the

cytoplasmic streaming inside cells (see Goldstein (2015) in the case of plant cells), while other internal fluids relevant to human health include synovial (joints) and cerebrospinal (brain and spinal cord) fluids.

In contrast, external problems are concerned with situations in which a fluid is located outside an organism of interest or its appendages and usually involve some degree of activity or deformation. The classical external problem is swimming (Childress, 1981), where the moving limbs of a human, or the fins of a fish, induce a flow in the surrounding fluid resulting in hydrodynamic stresses which, when integrated over the entire surface of the body, lead to a net propulsive force and locomotion. Another classical example is the impact of wind on plant growth (De Langre, 2008; Gardiner et al., 2016).

Clearly, the distinction between internal and external problems is not very precise. For example, the flow around a moving red blood cell is internal from the point of view of the organism whose blood is being examined, but external from the point of view of the cell. However, this distinction between two broad categories of problems has proven useful in grouping together biological systems involving fluid mechanics with similar dynamical regimes (Lighthill, 1975).

1.3 Biological Locomotion

The section of biofluid dynamics at the heart of this book is locomotion: How do living organisms explore a surrounding fluid and how are they constrained by it? Notably, the physical hydrodynamics of biological locomotion encompasses a large variety of problems with length scales ranging over seven orders of magnitude – from a few microns for cellular locomotion to tens of metres for the largest mammal. Accordingly, the dynamical regimes appropriate to capture all of biology include all of incompressible fluid dynamics, from the Stokes flow regime to turbulent boundary layers.

With this in mind, an alternative taxonomy is often proposed by fluid physicists to separate biological problems according to their relevant fluid dynamical regime (Childress, 1981). Consider a swimmer of size L propelling itself at speed U in a viscous, incompressible fluid of density ρ and dynamic viscosity μ. Ignoring unsteady effects, two timescales govern the motion of the fluid surrounding the swimmer. The first timescale, $t_1 = L/U$, is the typical time for a perturbation to the quiescent fluid to be advected along the swimmer. The second timescale, $t_2 = \rho L^2/\mu$, is the relevant diffusive timescale for a perturbation to the fluid to be dissipated away by the action of viscosity. Flows for which $t_1 \ll t_2$ are therefore dominated by advective transport whereas those where $t_1 \gg t_2$ are controlled by viscous effects.

Since both t_1 and t_2 have dimension of time, their ratio t_2/t_1 is dimensionless, and is in fact the relevant steady Reynolds number, Re, for the swimmer,

$$\mathrm{Re} \equiv \frac{\rho L U}{\mu}. \tag{1.1}$$

Locomotion problems tend to be understood according to the magnitude of their Reynolds number. Well-studied high Reynolds number locomotion problems include fish swimming, flying birds and insects. While very few organisms undergo locomotion in the intermediate regime, Re $\sim$ 1 to 10, another world exists where Re $\ll$ 1, the world of swimming microorganisms to which this book is devoted.

1.4 Locomotion at Low Reynolds Number

Microorganisms represent the bulk of the world's biomass, with over 10^{30} living cells, most of them prokaryotes. The ability to self-propel in a fluid, called motility, is a property of many cellular organisms, both prokaryotes and eukaryotes, and both unicellular and multicellular (Bray, 2000). The importance of local fluid motion to these organisms was long recognised but first modelled only in the 1950s, with the seminal work of Taylor and Lighthill at the University of Cambridge, and has since been at the centre of many studies at the intersection of applied mathematics, physics and biology.

The field has now reached a point where we understand the hydrodynamic framework required to model cell locomotion, and in particular the fluid dynamics of bacteria, plankton, spermatozoa and mammalian reproduction. In this book we cover the fundamental fluid dynamical modelling approaches for cell motility from the ground up. By focusing on a few model organisms, and showing systematically how a simple model may be constructed and solved, we are able to answer a series of biophysical questions of increasing complexity. Some model organisms considered repeatedly in this book are illustrated in Fig. 1.2, in a picture drawn to approximate relative scale. In order of increasing length scales of the cell body we tackle the propulsion of bacteria, spermatozoa, algae and ciliates.

1.5 Organelles that Confer Cell Motility

Before focusing on hydrodynamic aspects, we need to understand one important biological feature, namely the exact manner in which self-propelled cells create their swimming motion. All organisms studied in this book use slender whip-like organelles called flagella (singular, flagellum) in order to generate time-varying motion within the surrounding fluid. How do these organisms deform or move their flagella? Prokaryotes and eukaryotes have evolved fundamentally different solutions to this question, and while the same term of 'flagella' is used to denote

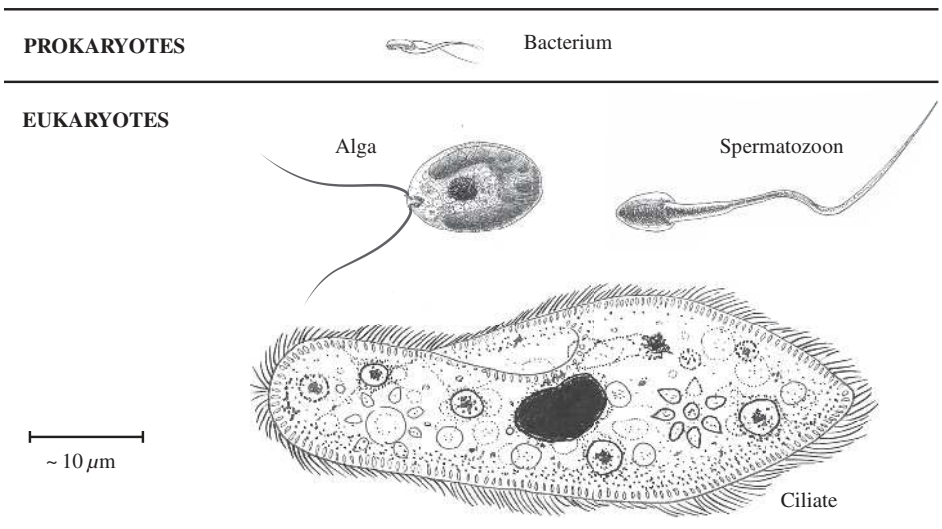

Figure 1.2 An illustration of the four model microorganisms that are studied in this book, drawn to relative scale; scale bar: 10 μm (approximate). Prokaryote: flagellated bacterium (model organism: *Escherichia coli*). Eukaryotes: flagellated spermatozoon (model invertebrate: sea urchin spermatozoon; model vertebrate: human spermatozoon); biflagellate alga (model organism: genus *Chlamydomonas*); ciliate (model organism: genus *Paramecium*). Drawings courtesy of Jacques Lauga.

the appendages of both, they are radically different in the manner in which they are molecularly actuated (Alberts et al., 2007). Both mechanisms, which were elucidated in the 1970s, are introduced briefly here and a more precise mathematical approach is offered in Chapter 5.

1.5.1 Prokaryotes

Prokaryotic cells (which we restrict here to mean bacteria) swim by rotating slender polymeric helices called flagellar filaments. The rotation of the filaments is driven by an intricate piece of molecular machinery called the bacterial rotary motor, a reversible stepper motor driven by ion fluxes, about 45 nm in diameter and able to generate rotation speeds of up to 300 Hz and torques of up to 5×10^3 pN nm (Berg, 2004).

The rotation of the motor is transmitted to the helices through a short flexible rod called the flagellar hook, which acts as a universal joint (see illustration in Fig. 1.3). Each helical filament is linked to a hook, which is actuated by an individual motor. The term 'flagella' then refers to the 'motor + hook + filament' apparatus. While some cells only have one motor, and thus actuate only one filament, many bacteria

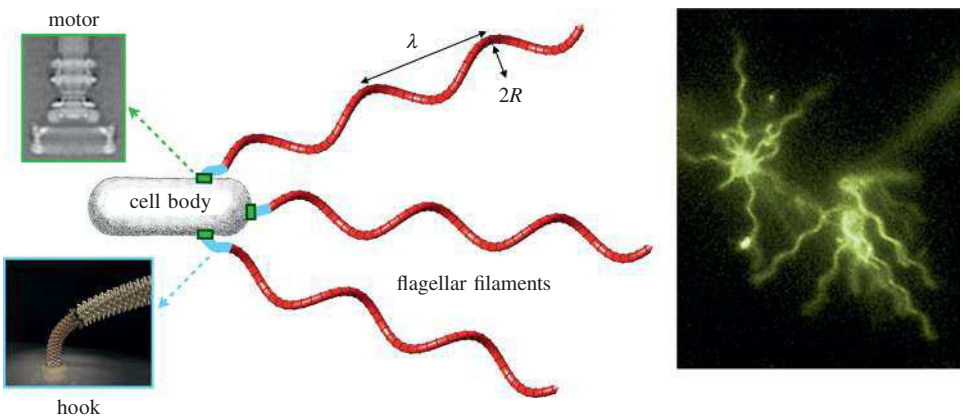

Figure 1.3 Left: flagellated bacterium where rotary motors embedded in the cell wall each use a short flexible filament called a hook. The hook acts as a universal joint to rotate passively a helical flagellar filament of pitch λ, which is on the order of microns, and radius R, which is typically hundreds of nanometres in size. Right: fluorescence microscopy picture of two *E. coli* bacteria displaying multiple identical flagellar filaments. Motor image reproduced with permission from Thomas et al. (2001). Hook picture courtesy of K. Namba, Osaka University. *E. coli* picture courtesy of Howard Berg, Harvard University.

employ multiple motors, each of them rotating an individual helical filament, as is the case for the oft-studied Escherichia coli (*E. coli*, Berg 2004). In all cases, locomotion of the cell results from the overall force and torque balance on the collection of rotating and interacting helices in the fluid.

The flagellar filaments, which are polymers made of a single protein conveniently called flagellin and all about 20 nm in thickness, are able to take one of 11 polymorphic forms depending on the chemical conditions and the behaviour of the cell body they are attached to (Macnab and Ornston, 1977). One of these forms is termed 'normal' and is used for forward locomotion by cells in their natural environment (wild-type cells); it has pitch $\lambda \approx 2.3\,\mu$m (i.e. the wavelength measured along the axis of the helix) and radius $R \approx 200$ nm (i.e. the radius of the cylinder on which the helix is coiled; see Fig. 1.3). During normal swimming, the normal flagellar filaments rotate at about 100 Hz with respect to the background quiescent fluid.

Importantly, from the point of view of the flagellar filaments the propulsion mechanism employed by bacteria is passive. During locomotion, each flagellar filament undergoes rigid-body rotation from an actuation point localised at one end (the motor). If one helical filament were to be cut in two, the half that is no longer connected to the hook would stop rotating and as such would no longer induce

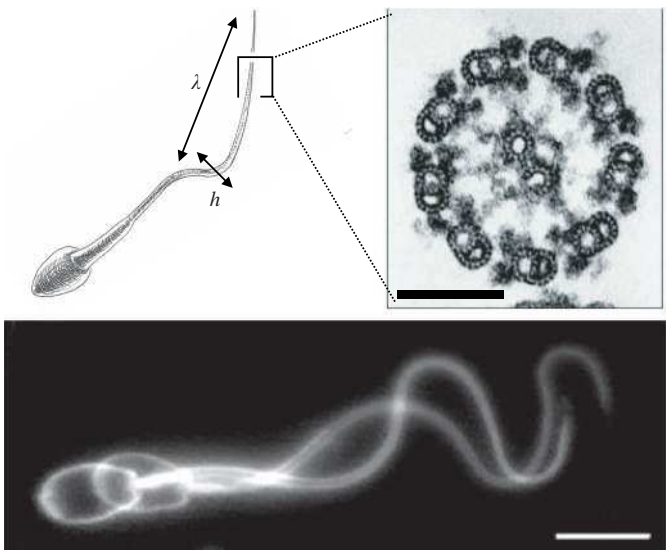

Figure 1.4 Top left: model spermatozoon swimming using active deformation of a flexible flagellum of wavelength λ, which is on the order of tens of microns, and amplitude h, which is typically a few microns. Drawings courtesy of Jacques Lauga. Top right: cross section of the internal structure of a flagellum (axoneme) showing the 9+2 structure of polymeric filaments (microtubule doublets); scale bar: 100 nm. Picture reproduced from Alberts et al. (2007). Bottom: picture of a ram spermatozoon at two times separated by half a beating cycle (0.28 s); scale bar: 10 μm. Picture used with permission of John Wiley and Sons from Woolley (2010); permission conveyed through Copyright Clearance Center, Inc.

locomotion. This passive mechanism for bacteria is to be contrasted with the active mechanism at the heart of eukaryotic swimming.

1.5.2 Eukaryotes

All swimming eukaryotes generate flows and propulsive forces from the actuation of flexible flagella. Despite carrying the same name as the prokaryotic apparatus, the 100 nm-thick eukaryotic flagella are instead similar to muscle fibres. Along an internal flagellar structure called the axoneme, long polymeric filaments (microtubule doublets) arranged in a 9+2 conformation as shown in Fig. 1.4 are made to slide past each other under the action of ATP-driven molecular motors (dynein). This sliding is converted geometrically into bending waves which propagate along flagella with wavelengths of tens of microns and frequencies on the order of tens of hertz.

While a range of flagellar arrangements exists, we address three prototypical situations in this book, as illustrated in Fig 1.2. The first is a model spermatozoon

(see also Fig. 1.4), where a cell body is propelled by a single flagellum undergoing approximately planar waving motion and pushing the cell forward. The second case is a biflagellate such as the green algae genus *Chlamydomonas*. These cells are pulled from the front by two flagella that often beat with mirror-image symmetry (Goldstein, 2015). The third example encompasses larger organisms called ciliates such as the protozoon *Paramecium*. Ciliates possess a large number of short flagella (given in this situation the appellation of cilia), whose asymmetric back-and-forth deformation strokes are coordinated in patterns of metachronal waves, allowing the cell to undergo three-dimensional helical motion.

It is significant to stress that, in contrast to prokaryotes, the actuation mechanism of eukaryotic flagella is active. The forcing from the molecular motors is distributed spatially along the entire flagellum. The molecular fuel, ATP, is produced by specialised organelles located in the thick portion of the flagellum, called the mid-piece, at the junction between the cell body and the rest of the flagellum, and diffuses along the axoneme. If a eukaryotic flagellum were to be cut in two, both halves would continue to beat, and self-propel, as long as they have access to ATP. Eventually, the half of the flagellum no longer connected to the cell body (and thus to the mid-piece) would run out of ATP and then stop beating. An important consequence of this active actuation mechanism is the time-varying deformation of the flagella. While bacterial flagellar filaments undergo rigid-body rotation, the shape of a eukaryotic flagellum changes in time, leading to a more complex fundamental balance of forces.

1.6 Cellular Locomotion as a Case Study in Modelling

Equipped with this biological background, we develop in this book mathematical models to capture the fluid-based locomotion of microorganisms and their interactions with relevant environments. We should keep in mind that this specialised topic represents but a small subset of all biofluid locomotion problems, which are themselves only a small portion of all external fluid dynamics relevant to biology, itself only a small part of the field of biological fluid dynamics.

By the end of this book, the reader will have learned how to exploit various modelling approaches to capture biological behaviour – from idealised two-dimensional models to flow singularities; from far-field interactions to near-field details. There remain vast and unexplored areas outside the scope of this book, and we hope that readers will adopt a similar attitude to model and understand many of these new problems.